

Failure of Proteolytic Enzymes to Suppress Post-Traumatic Inflammation

Double-Blind Control Comparison on Identical Twins, After Dermal Abrasion

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THIS IS THE FIRST controlled study demonstrating that systemic proteolytic enzyme therapy does not improve or modify posttraumatic edema, inflammation or speed of healing. Identical twins were subjected to an identical, severely traumatic surgical procedure (dermal abrasion of the face) at the same time. All surgical and postsurgical variable factors were made as similar as possible. One twin received maximum therapeutic dosage of three proteolytic enzymes for one week after operation. The other received an inert vitamin. Careful comparison revealed no discernible difference in postsurgical course.

Proteolytic enzymes have been widely reported to reduce inflammation, and cause resorption of edema. This is said to stop pain immediately, and to speed healing. Dramatic serial color photographs of traumatic injuries fill lavishly lithographed promotional material sent to physicians. The enthusiastic articles which testify to their effectiveness in human beings are uncontrolled.^{5,6,7,8} Animal experiments, well controlled, prove beyond cavil the anti-inflammatory effect of proteolytic enzymes.⁴ However, in these experiments the quantities of enzyme used were exponentially larger than recommended for human use—from ten to over one thousand times the maximum human therapeutic dosage.⁴ Moreover, these quantities were given before production of the inflammatory response—not after, as in a clinical case. Hence the fibrin and other insoluble aggregates that subsequently form in an inflammatory zone incorporate enough proteolytic enzyme to speed their resolution. Such animal experiments are not at all analogous to the human clinical situation.

VARIABLES IN RESPONSE TO TRAUMATIC INJURY

For proper evaluation of the effect of proteolytic enzyme therapy on the postsurgical course of human patients three sets of variables must be considered:

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• Controlled reports on the successful use of proteolytic enzymes to suppress post-traumatic inflammatory response have been based on animal subjects that were given the drug before inflammation was provoked and in the equivalent of 10 to 1,000 times maximum human dosage. All previous favorable reports on human subjects were based on uncontrolled experiments.

In the present study, the first controlled human experiment, identical twins were identically treated with dermal abrasion of the face, to remove deep acne scarring. Using double-blind technique, one of them received "inert" parenteral and buccal vitamin medication. The other received maximum recommended therapeutic dosage of three proteolytic enzymes—streptokinase and streptodornase buccal tablets, and intramuscular trypsin. No differences in edema, inflammatory response, pain or speed of healing were noted.

1. *Type and Extent of Injury*: Traumatic injuries in humans are usually accidental and randomly inflicted. The resultant inflammatory response and healing vary according to the degree, depth and extent of damage. Injury can rarely be standardized enough for comparison between cases. Animal experiments utilize graded tissue insults, such as injection or implantation of irritants. Such provocative tests can be well standardized.

2. *Extrinsic Variables*: Widely variable in human patients are such factors as age, nutrition, the degree of tissue damage by previous or concurrent disease or by previous sun exposure, x-ray therapy and the like. They can easily be minimized in animals by use of litter mates brought up under identical conditions.

3. *Intrinsic Variables*: What may be called "inherent tissue diathesis" embraces such factors as the individual immunity, regenerative power, type of protoplasm, type of connective tissue, capillary fragility, keloiding tendency, autogenous allergy and response to infecting organisms. Subject to wide disparity in humans, they can easily be equated in animals by the use of homologous strains and a larger number of test subjects.

In the treatment of twins with dermal abrasion for the smoothing of acne scars the author had opportunity to resolve these variables for an evaluation of the effect of proteolytic enzymes on human subjects.

In dermal abrasion (plastic planing) the acne pitted face (4 to 5 per cent of the total skin area) is fixed to woody hardness by solid freezing of small areas with Freon 114 spray. While each area is frozen solid, the entire epidermis and upper third of the cutis in the area is ground away with a steel brush cutting head, revolving at 14,000 revolutions per minute.

After doing 5,000 planings, I believe my technique is standardized to a high degree. The amount of surface treated (for example, the full face) and the depth of abrasion can be closely duplicated from one patient to the next, which provides a means of quantitative and qualitative control of variables for purposes of comparison. Inflammation after planing is severe, as after any extensive abrasive injury, such as deep "skinning" of an elbow or knee. The second through the fourth days after the operation, edema is often so extensive as to swell shut the eyes. Healing, with dropping of the crusts, usually takes eight days.

As to extrinsic and intrinsic variables in human subjects, in preliminary controlled comparisons of many patients, I found I was not able to pick out by inflammatory response (or lack of it) which patients had received proteolytic enzymes. However, I considered it possible that beneficial effects of the enzymes were masked by some of the extrinsic and intrinsic variables already mentioned.

The new science of gemellology, the study of twins, suggested a perfect control, since in any twins who had always lived together in the same environment, all extrinsic variables are equated. Monozygotic (identical) twins are genetically the same person. This genetic identity equates intrinsic variables. When a pair of identical twins were referred to me for plastic planing, these facts were used to set up a controlled evaluation of proteolytic enzyme therapy.

REPORT OF CASES

The patients were 26-year-old white women, registered nurses, who had lived as inseparable companions in the same environment since birth. In their early teens, both had severe acne simultaneously. An identical gamut of conventional therapy—local medications, surgical drainage, ultraviolet light, vaccines and the maximum allowable amount of x-ray treatment had been tried simultaneously on both, without significant improvement. When first seen by me, the two women were not only identical

in appearance of face and figure, but even in the severe irregular pitted postacne scars on their cheeks, foreheads, chins and necks. They had identical severe multiple, large, almost confluent, deep sebaceous cysts on the face, neck and retroauricular regions, widespread coarse comedosis, and secondary infection in scattered, deep, large pustular cystic lesions, all superimposed on severe seborrhea of the face.

Both were prepared for operation by giving them, an hour before the procedure, identical oral dosage of dextro-amphetamine, 10 mg.; amobarbital, 60 mg.; phenobarbital, 60 mg.; acetylsalicylic acid, 650 mg.; meperidine (Demerol®), 50 mg.; meprobamate, 400 mg. The planing operation on the second patient was done 20 minutes after the first. Planing extended vertically from the anterior scalp hairline over the entire face and anterior neck to the level of the cricoid cartilage, and from the midline of the nose to 5 cm. lateral to the angles of the mandibles. Because scarring was very deep, planing was very deep. Initial heavy planing was done with an abrasive serrated steel cutting head. Uneven residual scarred or pitted areas were then "feathered out" peripherally with further planing, and visible high areas were ground down to produce optimum flattening. All junctions of planed and unplaned skin were "feathered out" lightly with a diamond fraise. The thin skin on the lower eyelids was planed with this instrument.

Continuous Freon 114 (Frigiderm®) freezing was done by an assistant, just ahead of the planing brush. Simultaneously trichlorethylene (Trilene®) inhalation anesthesia was self-administered by the patient. Anesthesia and patient cooperation were excellent.

I tried consciously to equalize the degree of planing, the instrument used, the degree of freezing and all other variables. This was made easier because the lesions were closely duplicated in the two patients. At the periphery of the involved areas around the sides of the neck and laterally over the jaws, acne cysts were sparse enough for a pattern to be discernible. After the planing had been done on one patient, it could be predicted where the second would have cysts of the steatocystoma type that were not visible on surface inspection.

As a final check, to prove that the two patients were indeed monozygotic twins and not superficially similar dizygotic siblings, a 5 mm. punch skin graft was taken from a pitted scar below the ear lobe on each patient, and inserted in the donor site of the opposite patient. There was mutual acceptance of the reciprocal grafts, with no graft rejection after 18 months follow-up. This is considered the most absolute proof of monozygosity.

CHOICE OF PROTEOLYTIC ENZYMES FOR TESTING

Trypsin was selected as the primary therapeutic enzyme. It acts by breaking peptide linkages on carboxyl groups of arginine and lysine.⁵ Although it has produced severe, even fatal, anaphylactoid shock,^{1,9} it is considered about three times as effective as chymotrypsin.² (Until recently considered incapable of producing anaphylactoid shock, chymotrypsin was used extensively despite its relative ineffectiveness, but late reports indicate that it can produce severe anaphylactoid shock, including permanent brain damage.^{2,3})

As a secondary enzyme preparation, a mixture of streptokinase and streptodornase buccal tablets was selected, since its mode of action is entirely different from that of trypsin. On internal administration, streptodornase is inactive. Streptokinase activates blood plasminogen to plasmin. Plasmin acts to lyse fibrin.⁵

It seemed that maximal therapeutic dosages of these highly touted enzymes should surely produce discernible improvement to justify the considerable risk of allergic reaction to them.

DOUBLE-BLIND PLACEBO CONTROL THERAPY

As soon as operation on the second twin was completed, an attending nurse was told to determine by flipping a coin which twin would get proteolytic enzymes. That twin was given crystalline trypsin solution containing 5 mg. per cc., 2 cc. to begin with and then 1 cc. once daily, intramuscularly. In addition buccal tablets, each containing 10,000 units of streptokinase and 2,500 units of streptodornase (Varidase buccal®) were given on a schedule of 2 tablets four times daily. The other twin was given the same volume of crude liver parenterally, and

vitamin A buccal tablets on the same dosage schedule. I gave identical instructions for dissolving the buccal tablets under the tongue. I did not know which twin received the enzymes—and still do not. The patients were not told that there was any difference in their therapy. I observed and photographed their postoperative course. Not I or the patients or the attending nurse could see any discernible difference in speed of healing, or degree of swelling. Both patients had identical severe inflammation and postsurgical edema. In both cases the crusts fell off at the same time—eight days. There was no subjective difference in pain.

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